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Comparison of Four Commercially Available Group B *Streptococcus* Molecular Assays Using Remnant Rectal-Vaginal Enrichment Broths

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Running title: Molecular GBS assay comparison

Key words: Group B *Streptococcus*; *Streptococcus agalactiae*; neonatal sepsis; neonatal meningitis; real-time PCR

This is the author's manuscript of the article published in final edited form as:
Relich, R. F., Buckner, R. J., Emery, C. L., & Davis, T. E. (2018). Comparison of Four Commercially Available Group B Streptococcus Molecular Assays Using Remnant Rectal-Vaginal Enrichment Broths. Diagnostic Microbiology and Infectious Disease. https://doi.org/10.1016/j.diagmicrobio.2018.03.012

ABSTRACT

The incidence of neonatal Group B streptococcal (GBS) disease has significantly declined since the widespread implementation of prenatal screening of expectant mothers for urogenital and gastrointestinal tract GBS colonization. Screening methods have evolved from exclusively culture-based approaches to more rapid and highly sensitive molecular methods. We chose to evaluate the performance of four commercially available GBS molecular tests for detection of GBS colonization using 299 antepartum rectal-vaginal specimens submitted to our laboratory for routine GBS screening. In 97% of instances, there was agreement between all three systems. When testing 1, 6, and 12 samples simultaneously, all methods performed comparably, but the ARIES® GBS assay required the least total hands-on time and the *illumigene*® Group B *Streptococcus* assay required the most hands-on time.

1. Introduction

Streptococcus agalactiae (Group B *Streptococcus* [GBS]) is a Gram-positive, catalase-negative, facultative anaerobe that is known to harmlessly colonize the urogenital and gastrointestinal tracts of humans. In susceptible hosts (e.g., neonates, pregnant women, and those with chronic medical conditions), GBS is a versatile opportunistic pathogen that is capable of causing a variety of diseases, including urinary tract, respiratory, wound, and central nervous system infections (Spellerberg and Brandt, 2015). GBS is the leading cause of neonatal morbidity and mortality in the U.S. and, according to the Centers for Disease Control and Prevention (CDC), approximately 10-30% of pregnant women are colonized with this bacterium (CDC, 2010; Schuchat, 1999); in our patient population, the GBS colonization rate is approximately 20%.

Determination of maternal GBS colonization status prior to labor and delivery is paramount for guiding appropriate intrapartum antimicrobial prophylaxis (CDC, 2010). Currently, recommendations state that all pregnant women, except those who have had GBS bacteriuria during their current pregnancy and those who have previously delivered a child who developed early-onset GBS disease, should be screened for rectovaginal GBS colonization. For the exceptions noted, intrapartum antibiotic prophylaxis without rectovaginal screening is indicated since GBS bacteriuria diagnosed at any time during the current pregnancy and instances of early-onset GBS infection in previously born neonates are known risk factors for GBS infections in current pregnancies (Verani et al., 2010; Schrag et al., 2002; Faxelius et al., 1988). Because of nearly universal implementation of maternal prenatal GBS screening in the U.S., the incidence of neonatal GBS infections has dramatically declined (e.g., ~80% reduction in early-onset infections). Effective GBS surveillance strategies entail collection of rectal-vaginal

specimens from pregnant women between 35 and 37 weeks of gestation (CDC, 2010; ACOG, 2011). Swab specimens are subsequently analyzed for GBS by cultivation-based and/or molecular methods. Despite its relatively high sensitivity, selective culture is slow, requiring up to 48 hours for result reporting (Nomura et al., 2006); however, culture remains indispensable in cases where an isolate is required for antimicrobial susceptibility testing (e.g., when an alternate antibiotic must be used for patients who are allergic to β-lactam antibiotics). Although this slow turnaround time is tolerable for routine surveillance, it is unsatisfactory when a result is needed much sooner, such as when testing must be performed during active labor. In addition, with the assistance of automated molecular GBS testing platforms, numerous samples can be tested simultaneously, allowing laboratory scientists to perform other tasks.

Currently, there are several molecular GBS testing platforms that are commercially available as either *in vitro* diagnostic products or as research-use-only systems, as classified by the FDA. These assays employ nucleic acid amplification or nucleic acid probes for detecting GBS-specific genetic markers in either enrichment broths and/or directly from patient specimens. Depending on the needs of the laboratory, systems are able to perform single tests if test volumes are relatively low or multiple tests simultaneously if test volumes are high. In addition, the hands-on time (HoT) of these assays vary from long to short, the latter accounted for by the almost total involvement of automation in the testing process. The HoT required for these tests is important since most laboratories are staffed by scientists who are required to carry out numerous tasks throughout their work shifts. In laboratories that process large volumes of GBS specimens, test systems that offer relatively hands-free usage and provide high sample throughput often help mitigate the effects of laboratory staffing shortages and increased workload burdens. However, in small laboratories, systems that require a longer HoT may be

acceptable especially if the test volume is low and the physical space required to accommodate large instrumentation is not readily available. The aim of this study was to compare the performance characteristics, the HoT, and the total turnaround time (TAT; HoT plus automation time [AuT]) of four commercially available GBS molecular diagnostic tests: the BD MAX™ GBS assay, the ARIES® GBS assay, the *illumigene*® Group B *Streptococcus* assay, and the Xpert® GBS LB assay using remnant, de-identified, antepartum rectal-vaginal specimens that were submitted to our laboratory for routine GBS screening. Table 1 shows a comparison of various aspects of these test systems.

2. Materials and methods

2.1 Specimens. Two hundred ninety-nine remnant antepartum rectal-vaginal swab enrichment broths were de-identified according to institutional review board-approved methods and were enrolled in this study. Rectal-vaginal swabs (BBL® CultureSwab™ Liquid Stuart, double swab; BD) were collected from women ranging in age from 16 – 42 years (median, 27 years) who were between 35 and 37 weeks of gestation.

2.2 Standard-of-care (SOC) analysis. Rectal-vaginal swabs were broken off into Lim broth (BD), and inoculated broths were incubated for 18 – 24 h at 35°C in ambient air. Following incubation, 15-µl aliquots of well-mixed enrichment broths were tested by the BD MAX™ GBS Assay (BD) according to the manufacturer's instructions. External positive and negative controls were assayed once per day.

2.3 Comparator testing. Following SOC analysis, all remnant GBS enrichment broths were stored at 4°C and were tested by all comparator assays within 36 hours of placement in storage and in accordance with the manufacturers' specifications. Aliquots of remnant enrichment broths were tested by the ARIES® GBS Assay, the *illumigene*® Group B *Streptococcus* Assay, and the Xpert® GBS LB assay. Prior to comparator testing, broths were vortexed for 5 s to create a homogenous suspension. Testing was repeated once if invalid results were obtained in the initial test.

For ARIES® GBS testing, 200-µl aliquots were removed and pipetted into the sample chambers of ARIES® GBS Assay cassettes. Loaded cassettes were analyzed by the ARIES® System, which automates nucleic acid extraction and purification, as well as real-time PCR-based detection of GBS target nucleic acids.

For *illumigene*® Group B *Streptococcus* Assay testing, 50-µl aliquots of well-mixed broths were added to *illumigene* heat treatment tubes containing 200 µl of *illumigene* control reagent. Subsequently, samples were vortexed for 10 s and incubated for 10 min at 95ºC. Fifty-microliter aliquots of heat-treated samples were next added to *illumigene* reaction buffer tubes and were vortexed for 10 s. Fifty-microliter aliquots of the resulting solution were pipetted into test and control chambers of *illumigene* test devices. Following inoculation, test devices were inserted into the *illumipro-10* instrument for nucleic acid amplification and detection.

For Xpert® GBS LB assay testing, broth-saturated swabs were used to inoculate test cartridges, which were subsequently analyzed using a GeneXpert XVI instrument (Cepheid). Like the BD MAX and ARIES systems, the GeneXpert system fully automates all steps of GBS nucleic acid extraction, purification, and target detection.

External positive and negative quality control samples were assayed in accordance with manufacturer recommendations.

2.4 Discrepant result resolution. Discrepant molecular GBS test results were arbitrated by bacterial culture and bidirectional nucleotide sequencing. For bacterial culture, 10-µl aliquots of GBS enrichment broth were inoculated onto nonselective sheep blood agar plates (TSA w/ 5% sheep blood; Remel, Lenexa, KS), which were subsequently incubated for 24 h at 35°C in 5% CO_2. Suspicious colonies (e.g., small, translucent colonies surrounded by narrow zones of β-hemolysis) were identified using the PathoDX® Strep Grouping latex agglutination kit (Remel, Lenexa, KS). For nucleotide sequencing, total nucleic acids were extracted from enrichment broths using the NucliSENS® easyMag® system (bioMérieux, Durham, NC). PCR using primers targeting GBS genomic sequences distinct from those targeted by the molecular assays compared in this study (Table 1) was subsequently performed and amplicons were sequenced by capillary electrophoresis on an Applied Biosystems 3130*xl* genetic analyzer (Applied Biosystems, Foster City, CA). Resulting nucleotide sequences were analyzed using the 3130*xl* Data Collection software (v3.1.1) and Sequencing Analysis software (v5.4). Sequences that were at least 200 bases in length, had a Phred quality score of ≥20 for at least 90% of the bases, and contained fewer than 5% ambiguous base calls were subsequently analyzed using BLAST (Altschul et al., 1997). Acceptable matches to BLAST reference sequences were those with >95% query coverage and identity, and an E-Value <10-30 when compared to the reference sequence.

2.5 Hands-on-Time (HoT) and total turnaround time (TAT) analyses. For HoT and total TAT, the assay set-up, run initiation, analytical, and post-analytical times were measured using a stopwatch. HoT was evaluated for individual samples as well as when 6 and 12 samples were run simultaneously. Because the *illumigene*® system can only accommodate a maximum of 10 samples at once, 2 runs were performed in order to accommodate 12-sample testing. The data from these runs were combined. In order to calculate the total TAT, the HoT and the time samples were being analyzed by automated instrumentation (AuT) were added together. Data represent the average of two independent observations of testing performed by a single technologist.

2.6 Statistical analysis. Positive and negative percent agreement (PPA and NPA, respectively), in addition to overall percent agreement, along with confidence intervals, were calculated for each comparator assay using the BD MAX™ GBS Assay result as the non-reference standard. Calculations were performed according to U.S. Department of Health and Human Services guidelines (U.S. Food and Drug Administration, 2017).

3. Results

Of the 299 specimens included in this study, 147 (49.2%) were positive by all four platforms while 144 (48.2%) were negative by all. The percent GBS-positive, and Positive and Negative Percent Agreement results for each assay (as well as results of temporal aspect comparisons) are shown in Table 2. One invalid result was obtained by the ARIES® method; however upon retesting, a valid negative result was obtained. Nine of 299 (3%) specimens yielded discrepancies between methods. Resolution of discordant results is shown in Table 3.

The total HoT for performance of single samples was less than 5 minutes (range, 00:01:55 – 00:03:32 [h:min:sec]) for all assays. For a single sample, the ARIES® and Xpert assays both had HoT of 00:01:55, and the BD MAX and *illumigene* assays had HoT of 00:03:05 and 00:03:32. Results for assay runs of 6 and 12 samples are shown in Table 2.

The result TATs varied slightly for each assay when 1, 6, and 12 samples were run, but the overall TATs compared between methods ranged from less than 01:12:00 (h:min:sec) for the *illumigene* platform to approximately 2 h for both the ARIES® and BD MAX systems. None of the systems were interfaced but the time to print and enter results into the Laboratory Information System was measured at 4.2 minutes for 12 specimens. Results of total TAT analysis are compared in Table 2.

4. Discussion

The introduction of molecular methods such as real-time PCR and LAMP technologies has revolutionized the detection of GBS colonization. In addition to rapid result turnaround, the sensitivity of these methods is higher than that of culture alone. In a previous study that compared the performance of the BD MAX™ GBS Assay, the *illumigene*® Group B *Streptococcus* Assay, and the BD GeneOhm™ StrepB, all three assays demonstrated higher sensitivity than culture, which ranged from 67% to 73% (Couturier et al., 2014). A similar study that compared the BD MAX™ GBS Assay, the *illumigene*® Group B *Streptococcus* Assay, and the AmpliVue® GBS Assay (Quidel) found that these assays were between 37.3% and 46.4% more sensitive than culture for the detection of GBS colonization (Miller et al., 2015). In both of these studies, the agreement in sensitivities between platforms was high: 97.1% to 98.4% for Couturier et al. and 90.9% to 100% for Miller et al.

In this study, we sought to evaluate the performance characteristics, including the PPA

and NPA, and compare the temporal aspects of assay performance, including the HoT and total TAT, of four commercially available GBS molecular diagnostic tests for the detection of GBS colonization in antepartum women. To do so, we enrolled 299 remnant Lim enrichment broths originating in our laboratory from SOC antepartum GBS screening tests in a study that compared our in-house method, the BD MAX™ GBS assay, to three comparator methods, the Luminex ARIES® GBS research-use-only assay, the Meridian Biosciences *illumigene*® Group B *Streptococcus* assay, and the Cepheid Xpert® GBS LB assay.

The ARIES® GBS assay required the least total HoT for testing 1, 6, and 12 samples while the *illumigene*® Group B *Streptococcus* assay required the most HoT when testing 1, 6, and 12 samples. The fastest total TAT was seen with the Xpert® GBS LB assay, which required less than 01:12:00 minutes from sample manipulation to result reporting for 1, 6, and 12 samples. The slowest total TAT was seen with the BD MAX™ GBS assay when 12 samples were tested (time, 02:10:26). All systems tested in this comparison study produced actionable results much sooner than traditional, culture-based methods, which supporting the notion that molecular GBS testing is an ideal solution for GBS surveillance in modern clinical microbiology laboratories.

The analytical results from the four molecular methods was very similar and in agreement for 291 (97%) of the specimens tested. Overall, each of these broth-enrichment-based methods agreed with the culture and sequencing results 98% or more of the time, which is consistent with the information provided in each of the products' package inserts. In addition, discrepant resolution by bacterial culture and nucleotide sequencing detected GBS in two samples that the non-gold standard reference (BD MAX™ GBS) declared negative but one or more of the comparator methods declared positive, validating the importance of arbitration. As newer methods for the detection of maternal GBS colonization become available, similar studies

will be required in order to determine which system or systems fit with a laboratory's staffing and workflow requirements and other diagnostic testing needs. In addition, studies comparing the efficacy of current and future systems for the detection of GBS directly from clinical specimens are also needed.

Acknowledgments

We thank the Indiana University Health Division of Clinical Microbiology for their help with procurement of the remnant clinical material used in this study.

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TABLE LEGENDS

Table 1. Comparison of the GBS molecular assays evaluated in this study.

Table 2. Performance characteristics of the GBS molecular assays evaluated in this study.

Table 3. Results of discordant analysis for discrepant samples.

Table 1

Test	Company	Technology	Target	Regulatory Status	Approved specimen type(s)	Sample to answer?	Reported approximate assay turnaround time	Instrumentation and test capacity per instrument
BD MAX™ GBS Assay	Becton Dickinson, Sparks, MD	Real-time PCR using Scorpions® probes, qualitative	*cfb* gene, 124-bp amplicon	*In vitro* diagnostic	Lim Broth culture of vaginal-rectal swab specimens from antepartum pregnant women, incubated ≥ 18 hrs	Yes	<3 hrs	BD MAX™ System; 24
ARIES® GBS Assay	Luminex Corporation, Austin, TX	Real-time PCR using MultiCode®-RTx chemistry, qualitative	Genomic region downstream from the *cfb* gene	*In vitro* diagnostic[a]	Lim Broth culture of vaginal-rectal swab specimens from antepartum pregnant women, incubated 18-24 hrs	Yes	<2 hrs	ARIES® Systems; 6 and 12
illumigene® Group B *Streptococcus* assay	Meridian Bioscience, Inc., Cincinnati, OH	Loop-mediated isothermal DNA amplification	213-bp genomic region	*In vitro* diagnostic	Lim, TransVag, or Carrot Broth culture of	No	<1 hr	***illumi****pro-10*; 10

		(LAMP), qualitative			vaginal-rectal swab specimens from antepartum pregnant women, incubated 18-24 hrs			
Xpert® GBS LB assay	Cepheid Inc., Sunnyvale, CA	Real-time PCR using fluorogenic probe detection, qualitative	Genomic region adjacent to the *cfb* gene	*In vitro* diagnostic	Lim Broth culture of vaginal-rectal swab specimens from antepartum pregnant women, incubated 18-24 hrs	Yes	35-55 min	GeneXpert Systems; 1, 2, 4, 16, 48, and 80

[a]Test was Research Use Only (RUO) at the time of this study.

Table 2

Assay	GBS-Positive Samples (%)	PPA[a] (95% CI)	NPA[b] (95% CI)	HoT[c] (h:min:sec)			Total TAT[d] (h:min:sec)		
				1 specimen	6 specimens	12 specimens	1 specimen	6 specimens	12 specimens
ARIES® GBS	151/299 (50.5%)	98.7% (94.9% - 99.7%)	98.0% (94.1% - 99.3%)	00:01:55	00:04:45	00:08:42	01:56:43	01:59:49	02:04:06
BD MAX™ GBS[e]	150/299 (50.2%)	n/a	n/a	00:03:05	00:10:22	00:19:07	01:52:16	02:00:31	02:10:26
Illumigene® GBS	149/299 (49.6%)	98.0% (94.1% - 99.3%)	97.3% (93.2% - 99.0%)	00:03:32	00:11:41	00:18:25	00:53:32	01:01:41	02:03:23
Xpert® GBS LB	151/299 (50.5%)	99.3% (95.5% - 99.9%)	98.7% (94.9% - 99.7%)	00:01:55	00:07:25	00:14:00	00:56:59	01:02:44	01:02:44

[a]Positive Percent Agreement
[b]Negative Percent Agreement
[c]Hands-on Time
[d]Turnaround Time
[e]Standard of care method, used as comparator for this study

Table 3

BD MAX™ GBS	ARIES® GBS	*Illumigene*® GBS	Xpert® GBS LB	Adjudicated Result[a]
Negative	Negative	Positive	Negative	Negative
Negative	Negative	Positive	Negative	Negative
Negative	Positive	Negative	Negative	Negative
Negative	Positive	Positive	Negative	Positive
Negative	Negative	Negative	Positive	Negative
Negative	Positive	Positive	Positive	Positive
Positive	Negative	Negative	Negative	Positive
Positive	Positive	Negative	Positive	Positive
Positive	Positive	Negative	Positive	Positive

[a]Discordant results were adjudicated by culture and PCR with bidirectional sequencing.

BD MAX™ GBS	ARIES® GBS	*Illumigene*® GBS	Xpert® GBS LB	Adjudicated Result[a]
-	-	+	-	-
-	-	+	-	-
-	+	-	-	-
-	+	+	-	+
-	-	-	+	-
-	+	+	+	+
+	-	-	-	+
+	+	-	+	+
+	+	-	+	+

[a]Discordant results were adjudicated by culture and PCR with bidirectional sequencing.